

The Role of Olfaction in Courtship Behavior of the American Lobster *Homarus americanus*

DIANE F. COWAN

*Boston University Marine Program, Marine Biological Laboratory,
Woods Hole, Massachusetts 02543*

Abstract. Courtship behavior is well documented in captive lobsters. Sex pheromones contained in *female* urine and perceived by receptors on *male* antennules are thought to act as sex attractants or as signals necessary for pair formation. In this study, the lateral and medial antennules of male and female lobsters were removed. The result of these excisions were meant to indicate the gender-specific role of olfactory chemoreception in lobster courtship behavior. Removal of *male* antennules had little effect on pair bonding and mating. In contrast, removal of *female* antennules resulted in dramatic aberrations in behavior, including postmolt injuries and, in extreme cases, unsuccessful couplings and mortality. Therefore, *female* olfaction plays the more critical role in the normal reproductive behavior of *Homarus americanus*.

Introduction

Previous research on crustacean sex pheromones has focused on chemical signals found in female urine and detected by chemoreceptors on male antennules (see Dunham, 1978, 1988; Salmon, 1983, for reviews). For example, in courtship of the blue crab, *Callinectes sapidus*, females produce sex pheromones that are detected by receptors found on male antennules (Gleeson, 1980, 1982, 1984). In addition, many moth (and other insect) pheromones that act as sex attractants are produced by females and perceived by receptors on male antennae (Schneider, 1969; Conner *et al.*, 1980). Male pheromones play a role later in moth courtship, after the male has found the female, but before copulation (Eisner, 1980; Conner *et al.*, 1981). Because lobsters (*Homarus americanus*) are phylogenetically related to these organisms, they are assumed to have similar communication capabilities (Atema and

Engstrom, 1971). To understand the role of animal signals, the behavioral context in which a signal is used should form the basis of experimental design. Before molting, mature female lobsters choose a dominant male and cohabit in his shelter for several days before and after female molting. Mating occurs shortly after the female completes ecdysis.

In this study, the behavior of normal lobsters was compared to the behavior of antennule-excised lobsters. Both females and males underwent antennule excisions; control groups for both were also studied. In the *female excision experiment*, the antennules of female lobsters were removed while those of males were left intact; the results should elucidate the role of female olfaction in lobster courtship and mating behavior. If males must recognize the scent of sexually mature premolt females before “allowing” the females to enter their shelters, then pair bonding should occur between normal males and antennule-excised females, provided that females continued to produce the proper signals. If, however, females must recognize the scent of a suitable mate (dominant male) before entering the shelter, then females without antennules should either fail to pair bond or pair indiscriminately with dominant or subordinate males. If no differences in behavior occurred, we could conclude either that female olfaction is not essential for normal courtship behavior or that other chemoreceptors compensate for the loss of olfaction.

In the *male excision experiment*, the antennules of male lobsters were removed while those of females were left intact, to answer the following questions. What role, if any, does male olfaction play in pair formation and courtship behavior? Will antennule-excised males “allow” premolt females to share their shelters? Finally, a comparison of the two types of experiments should indicate

whether antennule-excision operations cause similar aberrant behaviors regardless of sex.

Because it is the female lobster that selects a mate and moves into his shelter, my working hypothesis was that removing male antennules would have little or no effect on pair formation. If antennule-excised males behaved normally, "permitting" unexcised premolt females to enter and cohabit in their shelters, this result would not only serve as a control for the female excision experiments, but would challenge existing hypotheses concerning the role of female sex pheromones and the importance of pheromone detection by male antennules.

Materials and Methods

Male and female lobsters were captured in the wild and all were handled identically. They were removed from holding tanks, measured, sexed, given individual identification marks, and assigned to groups. Each group of lobsters was composed of five sexually mature females and two sexually mature males ($\bar{X} = 80.4 \pm 2.44$ SD mm in carapace length) held communally in one of three 5600-l aquaria. No individual was used more than once.

The *female excision experiment* consisted of three groups of lobsters each comprising five antennule-excised females and two unexcised males. In one of the antennule-excised female groups no females molted, but molting is a prerequisite for pairing and mating. Therefore, only 2 of the excised female groups, totalling 10 females and 4 males, were included in the data. The *male excision experiment* consisted of two groups of five normal females and two antennule-excised males. The *unexcised controls* consisted of three groups, totalling 15 females and 6 males.

The excision operation was straightforward: the antennules were snipped off at the base with a pair of sharp scissors. Other experimental conditions were similar to those described in an earlier study involving normal lobsters (Cowan and Atema, 1990). Aquaria were provided with ambient flow-through seawater and a seasonally adjusted light/dark cycle. Live prey items were available at all times, and diet was supplemented *ad libitum* with an excess of freshly killed or frozen squid, fish, and clams.

The control experiments were carried out during June–September 1983 and May–November 1987, and some of these data have been published elsewhere (Cowan and Atema, 1990). Data from antennule-excised lobsters were collected during July–November 1990 and May–July 1991.

During censuses (at approximately 0600, 1200, 1800, and 2400, daily) the following information for each lobster was recorded: time, date, time of feeding, position and activity, indications of molt stage or (in females) egg carrying, location and condition of pieces of molt shells. Activities recorded included social interactions, feeding,

grooming, walking, resting, position of body and appendages, pushing gravel, digging, moving rocks, and building or repairing shelters.

Focal observations concentrated on each animal for 10 min twice a day, between 0700 and 0900, and between 1800 and 2100, whenever possible. At times, poor visibility made these observations impossible. Information was therefore quantified based on census rather than focal observations. Times of day for focal observations were chosen because female molting and mating usually occur in the morning (Cowan, pers. obs.), and the active period typically begins in the evening. In the aquaria, however, activity patterns are dependent on both the condition of the animal (*i.e.*, molt stage, egg bearing) and the time of day (Cowan, in prep.).

Long-term observations, focusing on pre-, post-, and molting females, were recorded in a notebook and on videotape. A light-sensitive video camera with a silicone intensifier tube was used to make video recordings at night. A color hand-held video camera was used during the day.

Duration of cohabitation for all molting females was determined during census observations. All cohabitation information is given as mean days \pm SD. Cohabitations were divided into periods of intermittent and permanent, pre- and postmolt shelter sharing, where "intermittent" indicates that the female was inside of the male shelter during at least one, but not all, of the census observations, and "permanent" indicates that the female was never seen outside of the male shelter on a given day. The day of molting was considered as part of postmolt cohabitation, because females usually molt in the morning.

When the observations had been completed, the seminal receptacles of the postmolt antennule-excised females were dissected and examined for the presence of spermatophores. The lengths of any regenerating antennules were also measured at the end of each experiment.

Because females usually mate with dominant males, male dominance was measured by tallying all observed male-male agonistic encounters. The animal that avoided contact, retreated, fled, backed away, or tail flipped was considered the subordinate of the pair in each group.

Results

Control lobsters

Thirteen of the 15 females chose mates and cohabited with males. The average duration of cohabitation was 12.9 ± 5.0 days (Fig. 1). After a brief period of precopulatory shelter sharing ($\bar{X} = 6.3 \pm 3.2$ days), each female molted inside of a dominant male's shelter. Males did not touch females during ecdysis. When each female gained enough skeletal support to stand up (usually 30 min after molting), she approached and turned so that her abdomen faced the male. The male then mounted and the pair copulated.

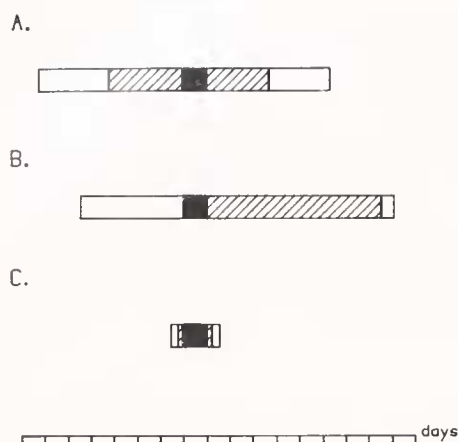


Figure 1. Mean duration of intermittent (open bars) and permanent (hatched bars) cohabitations between female and male lobsters *Homarus americanus*, relative to the day of female molting (filled bars). A, control ($n = 15$); B, normal females housed with antennule-excised males ($n = 10$); C, antennule-excised females housed with normal males ($n = 10$). In A, data are based on 13/15 females who molted and cohabited with males. In B, data are based on 3/10 females who molted and cohabited with males. In C, data are based on 6/10 females who molted, only two of which cohabited with males.

Each pair mated only once. After copulation, the females remained in the male shelter for 5.5 ± 3.2 days before leaving.

All thirteen females chose the dominant male as a mate; the remaining two females did not molt, cohabit, or mate.

Female antennule excisions

Antennule-excised females rarely pair bonded or cohabited. Six of ten antennule-excised females molted. Two of these six females cohabited briefly. The mean cohabitation duration for antennule-excised females was 1.0 ± 1.7 days (Fig. 1). Significantly fewer of the molting antennule-excised females cohabited as compared to controls (Fisher's exact test, $P = 0.02759$).

Post-treatment dissections revealed that five of the six molting females had mated (spermatophores present). Mating was observed in two cases. Both females were inseminated by the dominant male of their group.

The six females that molted showed a number of behavioral deviations never seen in controls. Four of these females molted outside their shelters and did not cohabit with males. One molted at night and without the protection of a male. She was killed after mating (spermatophore found in her spermathecum). A second did not mate. Two other females mated, but did not cohabit with males.

Of the two females that cohabited with males, one did so for two days after molting, and the other for two days before and two days after molting.

The deviations from normal behavior made copulation (which occurred at irregular times and places) difficult to

observe. But the two matings that were observed (one in each group) were between the females and the dominant male.

Finally, five of six molting females suffered injuries as a consequence of molting; including one death. In contrast, only one injury was observed among the 13 control females that molted.

Male antennule excisions

Three females (of 10) molted. Antennule-excised males "permitted" all three premolt females to enter their shelters and to cohabit before, during, and after molting (Fig. 1). The duration of cohabitation was comparable to that of the unmanipulated lobsters ($\bar{X} = 13.3 \pm 3.2$ days versus $\bar{X} = 12.9 \pm 5.0$ days). Female behavior was similar to that seen in control females; *i.e.*, females pair-bonded, cohabited, and mated with antennule-excised males, in male shelters.

Subtle changes in male behavior were recorded. For example, antennule-excised males stood over females and touched them with pereopods and maxillipeds (chemoreceptor organs involved in taste), while the females were undergoing ecdysis. In no other instance did males touch females during ecdysis. In addition, two of the females were injured by their mate during premolt cohabitation. No other premolt injuries occurred in any males or females.

No agonistic encounters were seen between antennule-excised males. Therefore, the dominance status of the males could not be determined.

Discussion

A single sensory system—olfaction—plays an important role in pair formation in lobsters. In these experiments, antennule-excised females risked injury to themselves and, in two cases (one female did not mate, another was killed), missed an opportunity to reproduce. In contrast, normal premolt females paired, cohabited, molted, and mated with antennule-excised males. Antennule-excised males showed an insignificant deviation from the normal sequence of mating behavior. These findings contradict previous claims that olfactory detection of female sex pheromones by males is an essential part of lobster courtship behavior.

The possible existence of a female lobster sex pheromone was first suggested by Hughes and Matthiessen (1962) who wrote, "It appears as if the freshly molted female exerts a chemical attraction on the male." Their statement led to research designed to develop a behavioral bioassay that could be used to isolate and identify a female lobster sex pheromone. In the early 1970's, two laboratories tested the responses of male lobsters to female odors. McLeese (1970) reported that male lobsters preferred the odor of freshly molted females to odors of other conspe-

cific. Atema and Engstrom (1971) concluded that “. . . a chemical compound (sex pheromone) is present in the water of a newly moulted female lobster that suppresses agonistic behavior and induces courtship.”

Because mating follows female molting, attempts to identify a pheromone concentrated on physiological correlates of molting (McLeese *et al.*, 1977; Atema and Gagosian, 1973). However, efforts to isolate sex pheromones from female lobsters failed. To gain a better understanding of lobster courtship behavior, observations were made in large aquaria, where females initiated pair formation several days before molting and cohabited with a male before and after mating (Atema *et al.*, 1979; Cowan and Atema, 1990). Adult lobsters did not cohabit under any other circumstances. Contrary to the existing hypotheses requiring *males* to recognize *female* odors before mating, the excision experiments reported herein show that *female* olfactory perception is critical for the successful completion of courtship and mating behavior.

Cohabitations

Female lobsters apparently benefit from choosing a mate able to protect them from predation and cannibalism during the vulnerable molting period. In this study, only 1 of 13 control females suffered a postmolt injury, while all of the antennule-excised female lobsters suffered postmolt injuries. In a separate experiment, females held together in the same aquarium without males all incurred serious postmolt injuries (Cowan, in prep.). Therefore, females benefit from male protection during postmolt cohabitation. Wilber (1989) showed that postcopulatory guarding by male crabs of the genus *Menippe* serves to protect the female from predators and to prevent extra-pair copulations. Shelter sharing in lobsters is analogous to mate guarding in many species of crabs that mate shortly after the female has molted (see Hartnoll, 1969).

The benefits of cohabitation appears to be greater for females than for males. Although males may benefit from cohabitations due to paternity assurance, males need not cohabit with females before inseminating them. Isolated females prevented from mating immediately after molting mated later when presented with males in communal tanks (Waddy and Aiken, 1990, 1991). The males discriminated between inseminated and uninseminated intermolt females and mated preferentially with uninseminated females (Waddy and Aiken, 1990, 1991). Female lobsters extruded fertilized eggs provided that insemination occurred at some time before egg extrusion, which often follows molting by many months (Waddy and Aiken, 1991; Cowan, in prep.).

Female antennule excisions

When deprived of their antennules, 4 of 6 females failed to cohabit. This failure to cohabit could be explained if

males produce odors that females must detect before entering male shelters for cohabitation. Or the communication system may be more complex. For example, females may need to smell males before producing female odors that the male detects before allowing females to enter their shelters and cohabit. Because most females were inseminated, males were apparently able to recognize sexually receptive, postmolt females. If male recognition of sexually receptive females is based on female odor production, these findings do not support the hypothesis that antennule-excised females failed to produce pheromones. Rather, the aberrations in the behavior of antennule-excised females were apparently due to the failure of females to detect male odors.

Unoperated-control females moved into the shelter of a dominant male and cohabited for several days before and after molting (see also, Cowan and Atema, 1990). Reliable information about male suitability as a mate may be contained in male odors detected by receptors on female antennules. If females cohabit with males because they detect male odors, then a failure to perceive male odors may lead to female rejection of males. Because females investigate male shelters from the outside, waterborne odors emanating from within may be difficult for females to detect without their antennules. The use of contact chemoreceptors for this function seems unlikely, because females do not have the opportunity to touch males with other chemosensory appendages during courtship. This would explain why most antennule-excised females failed to cohabit, especially if specific receptors that detect male odors are located only on female antennules.

Regeneration of female antennules may explain some of the variability in behavior shown by different females in this group. At 90-mm carapace length, lobsters normally have antennule flagella that are 40–50 mm in length. After excision, females began to regenerate their antennules; the longest regenerated antennule was 10 mm. Few aesthetascs were present on the regenerates.

In general, antennule-excised females with the longest regenerated antennules presented the most normal behavior. The female that molted 10 days postexcision did not regenerate her antennules and showed the most aberrant behavior. She molted outside of her shelter at night without the protection of a male and was killed shortly thereafter. Another female molted 34 days postexcision, regenerated 8.5 mm of her antennules, mated successfully, and was unharmed. The female who molted 46 days postexcision, regenerated 10 mm of her antennules and cohabited for 4 days: 2 days pre- and 2 days postmolt.

In contrast to this general trend, one female molted five days postexcision and cohabited intermittently for two days in spite of not regenerating her antennules. Her ability to cohabit cannot be explained by antennule regeneration. Perhaps this female had begun courtship before

her antennules were removed and was able to continue the sequence, although there was no premolt cohabitation. The effect of regeneration over a short time course is unclear.

Male antennule excisions

Unexcised females pair bonded, cohabited, molted, and mated with antennule-excised males. This indicates that male olfaction is not necessary for the normal sequence of lobster courtship behavior. How, then, is male signal detection or pheromone production involved in courtship?

Two pieces of evidence suggest that male signal detection was impaired by antennule excision. (1) There were no observed agonistic encounters between antennule-excised males. (2) An antennule-excised male wounded two mates during premolt cohabitation inside his shelter. The absence of male-male agonistic encounters suggests that male olfaction is involved in male dominance. The injuries inflicted on unexcised premolt females by antennule-excised males suggests that female odor may suppress male aggression toward females.

Atema and Engstrom (1971) hypothesized that females produce a pheromone that suppresses agonistic behavior and, therefore, protects soft-shelled females. Although two of the females were wounded during premolt, protection by the antennule-excised males during ecdysis suggests that other male chemosensory organs may have compensated for the loss of male antennules. Antennule-excised males touched the soft tissues of molting females with their pereopods and maxillipeds. These males may have been using gustatory chemoreceptors to compensate for the absence of olfaction. The same detection may not have been possible when antennule-excised males touched hard shelled females during premolt. This may explain why unexcised females who cohabited with antennule-excised males suffered pre- but not postmolt injuries.

Pheromone communication

Primer pheromones may function in the lobster mating systems by regulating the timing of female molting and subsequent mating (Cowan and Atema, 1990). In these experiments, fewer females molted when male antennules were excised and antennule-excised females molted with dominant males in sequence. However, the data reported here neither support nor contradict the primer pheromone hypothesis.

Odor communication appears to be very complex in this species. Successful courtship and mating probably involves the exchange of signals produced by both males and females. Perhaps the effects of antennule excisions were more dramatic in females than in males only because of the timing and method by which odors are transmitted.

Chemical signal detection by females is important during pair formation, whereas odor detection by males may be important later, at the time of female molting and shortly afterward when the pair mate.

Because females cohabit with dominant males, they are apparently able to discriminate male dominance. Perhaps females detect a male dominance odor which may also be important in intermale agonistic encounters. Some evidence for male sex pheromones already exists in Crustacea. Teytaud (1971) described particular behavioral changes in premolt pubertal female blue crabs (*Callinectes sapidus*) in response to male odors coupled with a visual model. His results showed that females detect and respond to male blue crab odors. More recently, Gleeson (1991) conducted choice tests in which pubertal female blue crabs showed a preferential attraction to male blue crab odors. Given a choice between male and female odor, or male odor and blanks, females chose male odor. However, females did not show a preference when given a choice between different males.

It has long been accepted that female odors and male olfaction play important roles in the reproductive behavior of Crustacea. The results presented here indicate that female olfaction also plays a crucial role in lobster courtship and mating behavior. Together with previous observations of lobster courtship behavior (Atema *et al.*, 1979; Cowan and Atema, 1990) and studies showing that females detect male odors (Atema and Cowan, 1986), these data show that female perception of male odors are of extreme importance in lobster reproductive behavior.

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